REMARKS

Revival

The present application, filed February 21, 1997, was deemed abandoned for failure to respond to the Office Action mailed September 16, 1997.

Applicants file concurrently herewith a Petition under 37 C.F.R. § 1.136(a), with fee, to extend time to respond to the outstanding office action to the full statutory period ending **March 16, 1998**. Applicants file concurrently herewith a Petition under 37 C.F.R. § 1.137(b) to revive the application for unintentional abandonment within one year of the expiration of the statutory period for response.

Applicants also file concurrently herewith an Associate Power of Attorney. Applicants invite the Examiner to telephone the undersigned, in whom Power of Attorney is newly vested, at Fish & Neave (650.617.4000) should any questions or concerns remain unanswered by the present papers; the correspondence address remains as previously directed.

As further discussed below, the claims of the present application stand provisionally rejected over both the claims and disclosure in parent application serial no. 08/760,447 ("the '447 application"), filed December 6, 1996. Applicants file concurrently herewith a Supplemental Declaration under 37 C.F.R. §1.67(a) that adds a claim under 35 U.S.C. § 120 to the benefit of the '447 application's filing date; the Supplemental Declaration brings the priority claim into conformity with the statement that appears on page 1, lines 6 - 7 of the present specification. Although the '447 application was copending at the filing of the present application, thus satisfying requirements for claiming benefit under 35 U.S.C. § 120, it has since been deemed abandoned for failure to prosecute. Applicants do not intend to revive

the parent application, thus obviating and mooting rejections in the present application that are provisionally based thereon.

The supplemental Declaration further adds Dr. Louis J. Picker, an inventor of the '447 application, as an inventor of the present application. A corresponding petition under 37 C.F.R. § 1.48(c) accompanies this response.

Information Disclosure Statement

Applicants invite the Examiner's attention to an Information Disclosure Statement filed concurrently herewith under 37 C.F.R. § § 1.56, 1.97(c) and 1.98.

Amendments to the Specification

In response to the Examiner's request, set forth at paragraph 2 of the outstanding office action, Applicants herein direct that a variety of amendments be made to the specification. Most of the amendments are self-explanatory and clerical in nature and clearly introduce no new matter. Two, however, merit additional comment.

Applicants request that "CD40", as it appears on page 5, line 8, be replaced by "CD40L". The passage in which the error appears discusses enhanced activation of T lymphocytes through use of antibodies or ligands that bind costimulatory molecules on the surface of T lymphocytes. The targeted costimulatory molecules interact with cognate antigens on the surface of accessory cells. Among the lymphocyte surface markers specifically so targeted are CD28 and VLA-4 (CD49d), both of which are known T cell surface antigens involved in T

cell activation. However, CD40, which is described in the same passage does not appear on the surface of T lymphocytes; the CD40 ***ligand***, denominated CD40***L***, is the costimulatory molecule on the T cell surface. Those skilled in the art would understand from the context that, like CD28 and VLA-4, CD40***L*** was intended. Because this typographical error would be recognized as such by those skilled in the art, and because the intended meaning would have been clearly and unambiguously understood, Applicants respectfully submit that the amendment adds no new matter.

Applicants also request that the word "IL-1", as it appears on page 10, line 5, be replaced by "IL-2". The reference to IL-1 occurs in the legend to Figure 3. The left-most panels of Figure 3 clearly show measurement of IL-2, not IL-1. Applicants respectfully submit that the amendment brings the description of the figure into conformity with the figure itself, and thus adds no new matter.

Amendments to the Claims

Applicants have canceled claims 1 - 18 without prejudice and add claims 19 - 60 by amendment herein. The claims newly added by amendment more particularly point out and distinctly claim Applicants' invention.

Claim 19, now the first independent claim in the application, recites as follows:

> 19. A method of detecting antigen-specific cytokine production by individual T lymphocytes, comprising the steps of:
> contacting a sample containing peripheral blood mononuclear cells with an MHC-dependent nominal antigen;
> adding to said sample an inhibitor of cytokine secretion;

adding to said sample at least one cytokine-specific antibody and at least one T lymphocyte subset-defining antibody; and then

flow cytometrically detecting the intracellular binding of said cytokine-specific antibody by cells in the defined T lymphocyte subset.

Claim 19 corresponds to, and finds support in, claim 1 as filed. Further support may be found in the specification as a whole, and particularly as follows.

An "MHC-dependent nominal antigen" is defined in the specification particularly at page 16, lines 5 - 11; page 4, lines 17 - 19; page 5, lines 19 - 21; page 8, line 25 - p. 9, line 7; p. 12, lines 1 - 4; page 5, lines 19 - 21; and page 16, lines 16 - 18.

The phrase "T cell subset-defining antibody" finds support in the specification at p. 12, lines 10 - 21; page 2, line 24 - p. 3, line 2; p. 5, lines 3 - 4; p. 7, lines 15 - 16; p. 8, lines 1 - 6; p. 8, lines 20 - 21; p. 9, lines 1 - 2.

Claims 20 - 22 correspond to, and find support in, originally filed claims 3 - 4. Support for costimuli of T cell activation may be found throughout the specification, and particularly at p. 5, lines 4 - 10; and p. 16, lines 16 - 18.

Language reciting "T lymphocyte early activation antigen" in claim 23 and its dependents finds support in claims 1 and 8 as originally filed, and in the specification particularly at p. 3, lines 7 - 11; p. 4, lines 20 -22; p. 5, line 3; p. 8, lines 15 - 18; and p. 16, lines 18 - 26.

The listing in claim 31 of types of MHC-dependent nominal antigens that prove useful in the present invention finds particular support in claim 5 as originally filed.

Support for the viral antigens particularly recited in claims 32 - 36 may be found throughout the specification, including the figures, and particularly as follows. Support for use of CMV antigens may be found, *inter alia*, at p. 8, line 24; p. 10, line 6; p. 10, line 26; p. 13, lines 7 - 11; FIGS. 1 and 2 and the legends thereto. Support for use of mumps antigen may be found particularly in FIG. 2 and the legend thereto; at p. 8, lines 23 - 25; p. 17, lines 13 - 15, and in claim 6 as originally filed. Support for the use of measles antigen may be found particularly in claim 6 as originally filed. References to HIV may be found throughout the specification.

Support for the recitation in claim 38 of *Mycobacterium* antigens may be found throughout the specification, and particularly in claim 6 as filed and at p. 10, line 26.

The cytokines specifically recited in claim 40 — IL-2, IL-4, IL-13, γ-IFN, and TNF-α — find support throughout the specification, in claim 7 as originally filed, and particularly in the specification at p. 3, lines 8 - 15; p. 5, lines 2 - 3; p. 7, line 10; p. 8, lines 15 - 18; p. 8, lines 23 - 25; p. 10, lines 3 - 6; and p. 18, lines 2 - 4. IL-2, IFN-γ, and TNF-α are specifically exemplified in FIG. 2.

The specificities of T lymphocyte subset-defining antibodies for CD3, CD4, CD8, TCR, homing receptors, CD45RO, CD45RA and CD27, referred to in claims

45 - 48, finds support *inter alia* at p. 12, lines 12 - 13; p. 5, line 4; p. 7, lines 15 - 16; p. 8, lines 2 - 5; and p. 9, line 2.

Claim 56's reference to "memory/effector T lymphocytes" finds support throughout the specification, and particularly at p. 1, lines 11 - 12; p. 1, lines 21 - 24; p. 2, lines 9 - 10; and p. 4, lines 24 - 27.

The phrase "immunomodulatory effects of a chemical compound," appearing explicitly in claim 58, and by dependency in claims 59 - 60, finds support particularly in claims 11 - 12 as originally filed; p. 4, lines 6 - 7; and p. 17, line 21 - p. 18, line 9.

The above discussion is exemplary, not exhaustive, of support for the claims newly added by amendment herein.

Color Figures

As suggested by paragraph 1 of the outstanding Office Action (paper no. 4, mailed September 16, 1997), the original of several of Applicants' drawings may have been rendered in color. Applicants hereby acknowledge that the requirements of 37 C.F.R. § 1.84(a)(2) must be met for formal acceptance of color drawings, and respectfully defer such action until examination concludes with indication of allowable subject matter.

Nomenclature

Because the CD ("cluster of differentiation") nomenclature was designed to identify human leukocyte antigens by the specificity of monoclonal antibodies commonly directed thereto, the CD designation may at times be used in the art to refer to the cell surface antigen that is bound by such antibodies or, in the alternative, to refer to the antibody itself. The distinction is typically clear from the context. To avoid ambiguity herein, the cell surface antigen will be referred to directly by its CD designation; an antibody specific for such a CD antigen will be denominated an "anti-CD" antibody.

Rejections Under 35 U.S.C. § 112, ¶ 1 for Lack of Enablement Are In Error And Should Be Withdrawn

The Examiner has objected to the specification and rejected prior-pending claims 1 - 18 under 35 U.S.C. § 112, first paragraph, "because the specification does not enable any person skilled in the art . . . to use the invention."

Cytokines

In a first rejection under § 112, the Examiner argues that "Applicant has not enabled measuring expression of one or more intracellular cytokines other

than γ-IFN, IL-2, IL-4, IL-5, IL-10 and TNF-α . . . [1] Applicants respectfully disagree.

> [I]t appears that these comments indicate nothing more than a concern over the breadth of the disputed term. . . . Accepting, therefore, that the term is a generic one, its recitation must be taken as an assertion by appellants that all of the "considerable number of compounds" which are included within the generic term would, as a class, be operative [in the invention]. . . . The only relevant concern of the Patent Office under these circumstances should be over the truth of any such assertion. <u>The first paragraph of § 112 requires nothing more than objective enablement. How such a teaching is set forth, either by the use of illustrative examples or by broad terminology, is of no importance.</u>
>
> As a matter of Patent Office practice, then, a specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented <u>must be taken</u> as in compliance with the enabling requirement of the first paragraph of § 112 <u>unless</u> there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support.

In re Marzocchi, 169 USPQ 367, 370 (CCPA 1971) (emphasis added); *quoted with approval* in *In re Brana,* 34 USPQ2d 1436 (Fed. Cir. 1995).

Applicants respectfully submit that the Examiner has advanced no such reason, theoretical or actual, to doubt the objective truth of Applicants' teaching that the invention may be practiced with

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[1] The Examiner's explicit recognition that Applicants have <u>indeed enabled</u> the detection of γ-IFN, IL-2, IL-4, IL-5, IL-10, and TNF-α precludes the assertion of this rejection to new claims 41 - 44, each of which is explicitly directed to one or more of the recited cytokines.

antibodies specific for other than the specifically recited and exemplified cytokines. Absent such reason, Applicants' teaching "must be taken as in compliance with the enabling requirement of the first paragraph of § 112."

The Examiner bears the initial burden of presenting a *prima facie* case of unpatentability; absent such *prima facie* case, the burden does not shift to Applicant to come forward with argument or evidence in rebuttal. *In re Oetiker*, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992). Applicants respectfully submit that no *prima facie* case has been presented. Nonetheless, solely to advance prosecution, Applicants respectfully direct the Examiner's attention to Exhibits A and B, attached hereto. Exhibit A, copied from the Becton Dickinson Immunocytometry Systems 1998 Product Catalog, lists fluorophore-conjugated antibodies sold explicitly for use in intracellular cytokine detection; among these antibodies are those specific for human IL-1α, IL-1β, IL-1ra, IL-2, IL-4, IL-6, IL-8, IL-13, IFN-γ, and TNF-α. Exhibit B, copied from the Pharmingen Cytokine/Chemokine Manual (2nd ed., April 1998), lists fluorophore-conjugated antibodies sold explicitly for use in intracellular cytokine and chemokine detection; among these antibodies are those specific for human IL-1α, IL--2, IL-3, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-16, GM-CSF, GROα, IFN-γ, IP-10, MCP-1, MCP-3, MIG, MIP-1α, RANTES, TNF-α and TNF-β.

Applicants respectfully submit that the commercial availability of these antibodies, sold explicitly for purposes of intracellular detection, bespeaks the applicability of the methods to a wide range of cytokines and chemokines, and would suffice to rebut any *prima facie* case to the contrary.

Applicants respectfully submit that the rejection under § 112, ¶ 1 is in error as regards claims 1 - 18, now canceled, and would for the same reasons be in error if asserted against claims 19 - 60, newly added by amendment herein.

Early Activation Antigen

In a second rejection under § 112, ¶ 1, the Examiner states that "Applicant has not enabled . . . measuring an early activation antigen other than CD69.[2] . . . The specification does not appear to specifically define the metes and bounds of . . . 'early activation antigen.'"

To the extent that the Examiner questions whether Applicants' specification enables the practice of the claimed methods across the scope of all early activation antigens, Applicants respectfully submit that the rejection is in error for the reasons advanced above, incorporated herein by reference: the Examiner has failed to provide reasons to doubt the objective truth of Applicants' teaching that the invention may be practiced with antibodies specific for other than the specifically recited and exemplified early activation antigens. The Examiner has failed to mount a *prima facie* case of nonenablement; Applicants' statements must be taken as in compliance with the enabling requirement.

It seems possible, however, that the Examiner's rejection is intended, instead, to question the

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[2] The Examiner's explicit recognition that Applicants have indeed enabled the practice of the claimed methods with antibody specific for CD69 should preclude the assertion of this rejection to claim 24, newly added by amendment herein, which is explicitly directed to use of anti-CD69.

definiteness of Applicants' terminology; that is, that the rejection instead questions whether the term "early activation antigen" is sufficiently well defined as to meet the requirements of § 112, ¶ 2; this would, of course, be a quite different rejection from that under § 112, paragraph 1.[3] If indeed the rejection is so intended, Applicants submit that the term has a clear meaning in the art.

Activation of T cells through the T cell receptor (TCR) complex initiates a number of well-characterized biochemical and morphological changes. These stereotypical changes culminate in T-cell differentiation and proliferation, and expansion of memory cells. One of the earliest changes noted is the expression of the activation antigen CD69, reaching peak expression within about 8 hours of stimulation. Other activation markers include CD25 (IL-2 receptor), CD71 (transferrin receptor) and HLA-DR, although their expression lags that of CD69.

Those skilled in the art recognize that "early activation antigens", as used in Applicants' specification and claims, refers to those cell surface proteins that reliably appear on the surface of T lymphocytes early in this stereotypical activation process. In the context of Applicants' assay, those skilled in the art would further recognize that only those activation markers expressed by the completion of incubation would usefully be within the scope of the term "early activation antigen." The term is clear, precise, and definite. No more is required.

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[3] *W.L. Gore & Assocs. v. Garlock, Inc.*, 220 USPQ 303 (Fed. Cir. 1983).

Co-stimulus

The specification teaches that "the antigen specific response can be more easily detected when the antigen stimulation is provided in conjunction with costimulation of surface antigens involved with accessory cell surface molecules, such as CD28, CD40L[4] VLA-4, and other such specificities known in the art." Specification p. 5, lines 5 - 8. Effective costimulation causes an increase in the percentage of T lymphocytes that may be seen to respond to a given MHC-dependent nominal antigen, as assessed using the methods of the present invention.

In a final rejection under 35 U.S.C. § 112, first paragraph, however, the Examiner expresses concern about the scope of the term "costimulus of T cell activation," commenting that Applicant has not "enabled . . . providing a costimulus other than via CD28, CD40, CD86 or CD118."[5] No further reason is given. For the reasons advanced above, these concerns do not rise to a *prima facie* case of nonenablement; the Examiner has not shown any reason to doubt the objective truth of Applicants' assertion that the invention may be practiced with other than an anti-CD28 antibody. Nor has the Examiner provided any reasoned statement as to why such costimulatory specificities may not readily be identified using the assay described in the specification.

Nonetheless, solely to advance prosecution, Applicants respectfully submit evidence showing that antibodies specific for VLA-4 (CD49d) and those directed to CD5 are effective as costimuli. As shown in Exhibit C

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[4] As amended.

[5] The rejection is thus inapplicable to claim 21, drawn with particularity to use of an anti-CD28 antibody.

attached hereto, 1.35% of peripheral blood $CD4^+$ T lymphocytes respond to CMV in the absence of costimuli; 1.86% respond when anti-CD28 mAb is included; and 1.73% respond when an anti-CD49d (VLA-4) antibody is included. Exhibit D shows similar results using an anti-CD5 antibody.

Applicants respectfully submit that as applied to claims 1 - 18, now canceled, the rejection is in error, and that the rejection would, for the same reasons, be in error if brought against claims 19 - 60, newly added by amendment herein.

Rejection Under 35 U.S.C. § 102(b) Is In Error And Should Be Withdrawn

The Examiner rejects claims 1 and 3 - 15 under 35 U.S.C. § 102(b) as anticipated by Picker *et al., Blood* 86: 1408 - 1419 (Aug 1995) ("Picker). The Examiner recognizes that claims 2 and 16 - 18 are free of anticipation.

Before addressing the substance of the rejection, Applicants wish to clarify that the standard under which anticipation is judged is rather more rigorous than might be suggested by the Examiner's brief comment that "no more of the reference is required than that it sets forth the substance of the invention."

"Anticipation requires the presence in a single prior art reference disclosure of each and every element of the claimed invention, arranged as in the claim," *Lindemann Maschinenfabrik GMBH v. American Hoist & Derrick Company,* 221 USPQ 481, 486 (Fed. Cir. 1984); "[i]t is axiomatic that for prior art to anticipate under 102 it has to meet every element of the claimed invention," *Hybritech inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81, 91 (Fed. Cir. 1986).

Claim 1, now canceled, is drawn to "[a] method for determining the antigen specific activation of T cells,"[6] comprising, *inter alia*, "culturing . . . with an antigen specific stimulus. . . . " (emphasis added). Claims 2 - 18, also cancelled, incorporate these elements by dependency. 37 C.F.R. § 1.75(c).

Picker does not disclose antigen-specific activation using an antigen specific stimulus. Rather, Picker discloses: (1) activation using the polyclonal mitogenic combination of phorbol 12-myristate 13-acetate plus ionomycin (PMA+I); and (2) activation using the bacterial superantigens staphylococcal enterotoxin B (SEB) and staphylococcal enterotoxin A (SEA).[7]

That claims 1- 18, which are directed to "antigen-specific activation" using an "antigen specific stimulus" are distinguished over Picker's use of PMA+I as a polyclonal mitogen should be self-evident: mitogenic stimulation is quite clearly independent of both antigen and accessory cell MHC.

That claims 1 - 18 are equally distinguished over Picker's use of superantigens warrants further comment.

Superantigens are proteins — typically derived from bacterial pathogens — that stimulate T cells. Like classical T cell antigens, superantigens physically bind

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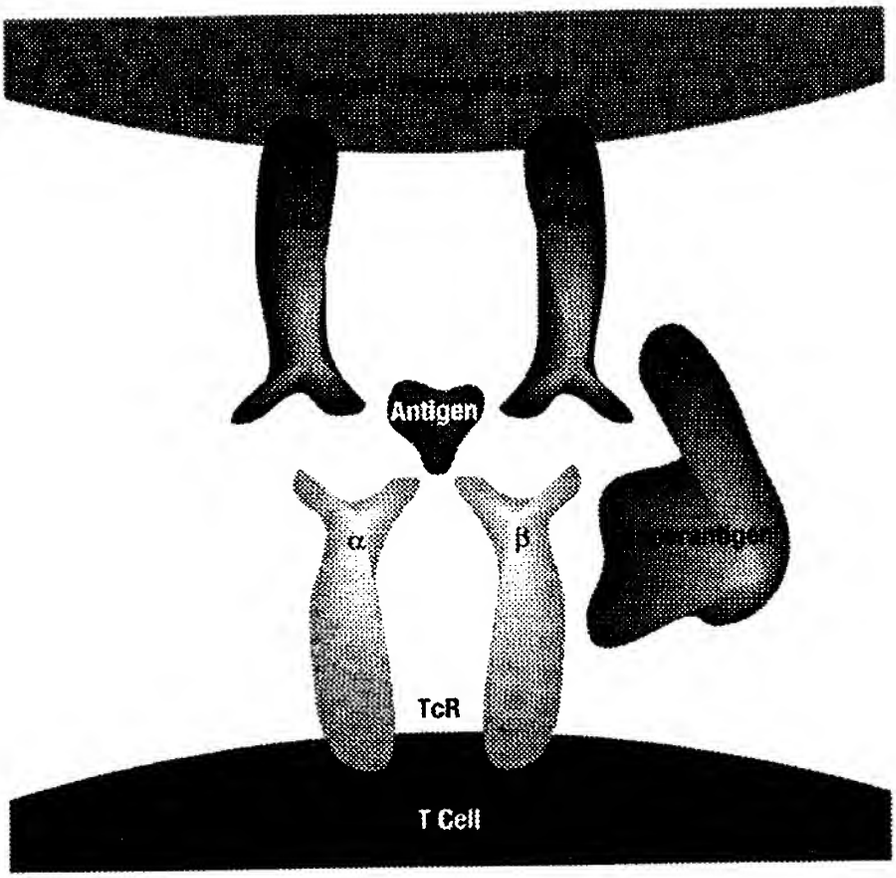
[6] The preamble is properly considered in assessing anticipation. *Diversitech Corp. v. Century Steps Inc.*, 7 USPQ2d 1315 (Fed. Cir. 1988).

[7] "Here, we improve on these [prior] methods and combine them with sophisticated multiparameter analysis techniques to simultaneously determine the cytokine production capabilities of precisely defined subsets of human peripheral blood . . . and inflammatory site T cells after only short periods (4 to 8 hours) of **in vitro activation with mitogen or superantigen**."

-- Picker, p. 1409, col. 1 (emphasis added).

to both the T cell receptor (TCR) and MHC class II determinants on the surface of antigen presenting cells. In contrast to classic MHC-dependent antigens, however, the superantigens bind outside the antigen binding site created by interaction of TCR Vα and Vβ.



Model of T-Cell Activation by Superantigen

As a result of this anomalous binding to Vβ, superantigens activate subsets of T cells that express the appropriate Vβ chain <u>independently</u> of the T lymphocyte's <u>antigen specificity</u>. SEA, for example, activates T cells bearing Vβ1.1, Vβ5, Vβ6, Vβ7.3, Vβ9.1; among these cells are represented a wide array of antigen specificities. SEB activates T cells using Vβ3, Vβ6,

Vβ12, and Vβ17, again representing a cross-section of the immune system's antigen-recognition repertoire.

Thus, Picker's use of superantigens as T cell activator cannot anticipate claims 1 - 18, for superantigens, which are not "antigen specific stimul[i]," do not occasion "antigen-specific activation." Claims 1 - 18 are not anticipated by Picker, and the rejection is thus in error.

In order to expedite prosecution, however, Applicants have reworded the corresponding clauses of the claims newly added by amendment, more particularly to point out and distinctly claim this element of the invention.

Thus, claim 19, now the first independent claim, recites, in relevant part: "A method of detecting antigen-specific cytokine production by individual T lymphocytes, comprising the steps of: contacting a sample containing peripheral blood mononuclear cells with an MHC-dependent nominal antigen. . . ." (emphasis added).

The term "nominal antigen" is explicitly defined in the specification as an antigen ***other than*** polyclonal mitogens or superAgs.[8] The specification further clarifies that the antigens of interest in the present invention are those that require presentation by

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[8] "Previous methods for detecting and quantitating T cell cytokine responses to polyclonal mitogens (e.g. phorbol ester plus ionomycin) and superAgs (the staphylococcal enterotoxin superAgs SEA and SEB) using a protocol based on intracytoplasmic staining of cytokine in short-term activated, secretion-inhibited T cells and multiparameter flow cytometric analysis has been previously shown. It was initially anticipated that, with slight modifications, this approach would identify and quantitate T cells producing cytokine in response to **nominal Ags *as well***."
--Specification, p. 16, lines 5 - 11 (emphasis added)

MHC.[9] Together, these terms affirmatively exclude the polyclonal mitogens and superantigens of the prior art.

Because "[a]nticipation requires the presence in a single prior art reference disclosure of each and every element of the claimed invention, arranged as in the claim," and because the claims explicitly and affirmatively recite elements that exclude the prior art, the claims newly added by amendment cannot be anticipated by that art. On that basis, Applicants respectfully request that the rejection of record, now obviated, would be in error if asserted against claims 19 - 60, newly added by amendment.

Rejections Under 35 U.S.C. § 103 Over Picker Are In Error And Should Be Withdrawn

The Examiner rejects claims 1 - 18 as unpatentable under 35 U.S.C. § 103 as obvious over Picker,[10] arguing that "[o]ne of ordinary skill in the art at the time the invention was made would have been motivated to select multi parametric flow cytometry analysis to determine antigen specific activation of T

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9 "The method described herein is composed of two parts. The first process relates to the cell culture process for optimal antigen presentation in MHC directed T cell responses (primarily but not restricted to memory CD4+ T cell responses)." Specification p. 4, lines 17 - 19.

10 Taken in view of "art known procedures to use cationic chelating agents in flow cytometry, as evidenced by Seon *et al.*, U.S. Patent No. 5,407,805," in view of "art known procedures to lyse red blood cells, as evidenced by Schwartz (U.S. patent No. 5,093,234," and in view of "art known motivation to detect antigen specific activation to a wide variety of antigens."

cells to a wide variety of antigens as a useful tool to analyze and characterize T cell immunity with a high degree of specificity and sensitivity. From the teachings of the references, it was apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention."[11] Applicants respectfully disagree.

As noted above, Picker neither discloses nor suggests the possibility of using antigen-specific stimuli to effect antigen-specific activation of T lymphocytes for purposes of subsequent flow cytometric analysis. Instead, Picker utilizes polyclonal mitogens or superantigens to stimulate relatively large percentages of T lymphocytes; the reason has to do with numbers.

Even after **near-universal** activation of T lymphocytes by polyclonal mitogen (PMA+I),[12] only **2.5**% of activated $CD8^-$ lymphocytes, and only **0.8**% of activated $CD8^+$ cells, can be shown to express IL-4. Analogously, after stimulation of **20.9**% of $CD4^+$ cells by superantigen only **1.8**% of the $CD69^+CD4^+$ cells can be shown to express IL-4 (Picker fig. 3). IL-2 and γ-IFN are produced in a

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11 "The consistent criterion for determination of obviousness is whether the prior art would have suggested to one of ordinary skill in the art that this process should be carried out and would have a reasonable likelihood of success, viewed in the light of the prior art. Both the suggestion and the expectation of success must be founded in the prior art, not in the applicant's disclosure." *In re Dow Chemical Co.*, 5 USPQ2d 1529 , 1532 (Fed. Cir. 1988).

12 Picker's figure 1 shows that, after 4 hours' incubation with PMA+I and Brefeldin A (BFA), fully **94.7**% of $CD8^-$ (that is, $CD4^+$ cells) become $CD69^+$, indicating activation; Picker's figure 1 further shows that **96.4**% of $CD8^+$ lymphocytes become $CD69^+$ after incubation with PMA+I and BFA.

higher percentage, but still only in a subset of the activated T cells.

Elsewhere, the prior art had suggested that **far fewer** T lymphocytes would be activated by an antigen-specific stimulus. Reports in the literature using limiting dilution assays (LDA) and enzyme-linked immuno spot (ELISPOT) assays had suggested that precursor frequencies for CMV-reactive and PPD-reactive T cells range from about 1/10,000 to 1/1000 mononuclear cells in appropriately exposed individuals, below the known detectable dynamic range for flow cytometric detection. Using LDA, for example, Brett *et al.*[13] report frequencies of peripheral blood T lymphocytes responsive to PPD between 1/13,982 (0.007%) and 1/1970 (0.05%); using ELISPOT, Lolli *et al.*[14] report median numbers of CMV-reactive T cells in HIV infected individuals of $8/10^5$ peripheral blood mononuclear cells (0.008%) and median numbers of PPD-reactive T cells of $4/10^5$ (0.004%).

In light of this prior art, Picker's results would have suggested that antigen-specific stimulation would not produce sufficient numbers of activated T lymphocytes to permit the detection of individual (single cell) cytokine production. There would have been neither motivation to attempt the experiment nor a reasonable expectation that the experiment would succeed.

In light of this prior art it is, in fact, quite surprising that the experiments reported in the

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13 Brett *et al.*, "Limiting dilution analysis of the human T cell response to mycobacterial antigens from BCG vaccinated individuals and leprosy patients," *Clin. exp. Immunol.* 68:510-520 (1987) (attached hereto as Exhibit E).

14 Lolli *et al.*, "HIV antigen-reactive T cells detected by antigen-induced interferon γ secretion," *AIDS Res. Human Retrovir.* 10:115 (1994) (attached hereto as Exhibit F).

present application worked at all. And the reason they worked is equally surprising: they worked because the LDA data and ELISPOT data in the prior art were **wrong**, underestimating frequencies of antigen-responsive cells by as much as 10- to 100-fold. This latter result would not have been predicted; indeed, the flaw in the time-honored prior art approaches was demonstrated by use of the methods of the present invention. But that, of course, is a story that properly resides in the "tempting but forbidden zone of hindsight,"[15] and need not be retold here.

Thus, Applicants respectfully submit that this alone demonstrates that all of Applicants' claims would have been nonobvious. The prior art neither suggested the methods of the present invention, nor would have predicted their operability.

Antigen-specific stimulation differs from mitogen and superantigen activation in yet another fundamental way: uniquely among these activators, nominal antigen requires processing by antigen presenting cells (APCs) prior to display on MHC molecules and lymphocyte triggering.[16]

Most protein antigens must be processed into short peptide fragments, noncovalently attached to MHC, and then displayed on the surface of an antigen presenting cell (APC) before triggering a T cell response. Class I-associated peptides are most often generated from endogenous cytosolic proteins. These endogenous proteins are cleaved and translocated into the lumen of the ER, where they are loaded onto MHC class I molecules that have been arrested therein. Charging with

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[15] *Loctite Corp. v. Ultraseal Ltd.*, 228 USPQ 90, 99 (Fed. Cir. 1985).

[16] Although superantigens require contact with APC MHC, no processing by the APC is required.

peptide completes the class I molecule's folding pathway and permits transport of the MHC/peptide complex to the Golgi. MHC class II-associated peptides, in contrast, are generally derived from extracellular proteins: processing of antigens for presentation by class II molecules involves the endocytosis, proteolysis and intracellular loading of the resulting peptides onto class II MHC before display on the APC surface.

Studies of the kinetics of class II-mediated antigen processing and presentation had suggested, as recently as 1996, that significant enzymatic degradation of an exogenous protein antigen does not occur until about 3 hours after endocytosis;[17] it had further been shown that up to 4 hours would be required to bind immunogenic peptides to class II molecules.[18] Thus, nothing in the prior art would have predicted that endocytosis, proteolytic processing, MHC presentation and T cell stimulation could be completed quickly enough to permit antigen-specific T cell activation to be assessed in a brief *in vitro* assay, as described and claimed in the present application.[19]

Nothing in Picker, surely, would have so predicted. To the extent that Picker touches upon issues that relate to processing and presentation of nominal MHC-dependent antigens, it teaches away from successful application of the method claimed herein: Picker teaches

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17 Weaver *et al.*, "Macrophage mediated processing of an exogenous antigenic fluorescent probe: time-dependent elucidation of the processing pathway," *Biol. Cell* 87:95-104 (1996).

18 Germain *et al.*, "The biochemistry and cell biology of antigen processing and presentation," *Annu. Rev. Immunol.* 11:403-450 (1993).

19 The experiments illustrated in FIGS. 1 and 2 of the present application were performed using 6 hour incubation with <u>whole virus</u> antigen.

that T cell activation by mitogens and superantigens may be observed even with concurrent incubation in the presence of Brefeldin A, an inhibitor of the protein secretory pathway.

> In preliminary experiments, BFA (10 µg/ml final concentration) completely blocked the surface appearance of the activation Ag CD69 on 4-hour PMA + I stimulated T cells without any loss of viability (viability maintained for up to 18 hours). **Importantly, BFA did not interfere with early activation events,** because the level of intracytoplasmic CD69 staining (see below) shown by PMA + I-activated, BFA-treated T cells was almost identical to the level of surface CD69 staining on non-BFA-treated, similarly activated T cells (data not shown).[20]

In contrast, the present specification teaches that "responses were maximized when Brefeldin A was omitted from the initial hour of interaction (likely to allow antigen processing). . . . "[21]

Thus, Applicants respectfully submit that neither motivation to modify Picker to attempt detection of antigen-specific responses nor reasonable expectation of successfully accomplishing this goal may properly be found in the prior art. The rejection of claims 1- 18, now canceled, is in error; Applicants respectfully submit that the rejection would equally be in error as applied to claims 19 - 60, newly added by amendment herein.

Claim 20 and claims dependent therefrom further require that a costimulus of T cell activation be added to the sample contemporaneously with antigen contact. As noted in the specification, costimulation increases the percentage of T cells responding to specific antigen with cytokine production. Applicants respectfully submit that

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[20] Picker, p. 1409, col. 2.

[21] Specification p. 16, lines 16 - 18.

this result – activation of increased numbers of T cells – would not, and indeed could not have been predicted, and that there would therefore have been neither motivation nor a reasonable expectation of success in adding such a costimulatory agent to the flow cytometric methods claimed herein.

The prior art teaches that costimulation should increase the cytokine-specific signal observed per activated cell – by increasing the stability of various cytokine mRNAs,[22] by reducing the rate of decay of IL-2 mRNA,[23] by increasing IL-2 gene transcription[24] – but does not necessarily predict an increased number of cells so activated. Inasmuch as detection is limited in the methods of the present invention by the number of positive events, not by the intensity of their staining, there would have been predicted to be no significant salutary effect of costimulation in the present methods.

Picker is not to the contrary, notwithstanding the Examiner's suggestion that "Picker et al.[,] . . . by teaching CD28, . . . would have led the ordinary artisan to apply other known T cell costimuli at the time the invention was made."

Picker uses a combination of anti-CD3 and anti-CD28 antibodies as yet another polyclonal mitogen, akin to PMA+I: the "[p]reliminary data . . . indicate that T-cell stimulation with anti-CD3 and anti-CD28 yields analogous cytokine-producing subsets. . . . These findings strongly suggest that, **in the setting of a fully activating stimulus**, the observed heterogeneity of

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22 June *et al.*, *J. Autoimmun.* 2 Suppl:55-65 (1989).

23 Umlauf *et al.*, *Mol. Cell. Biol.* 15:3197 (1995).

24 Williams *et al.*, *J. Immunol.* 148:2609 (1992).

memory/effector T-cell cytokine production is an intrinsic characteristic of the T cell. . . . [25]

Nowhere does Picker suggest the use of anti-CD28 with a stimulus that is **other than fully activating**. And in the setting of a fully activating stimulus, no suggestion that anti-CD28 might effect an increase in the number of activated T cells can be inferred. In light of the art-accepted teaching that costimulation should not increase the number of responding T cells, there would have been neither motivation to attempt the experiment nor reasonable expectation of success in so doing. Applicants respectfully submit that, for these additional reasons the invention set forth in claim 20 and claims dependent therefrom would have been nonobvious over the art of record, and that the rejection should be withdrawn.

Claim 26 is further directed to detecting antigen-specific cytokine production by individual T lymphocytes directly in a whole blood sample. The specification teaches that using whole blood, as opposed to fractions enriched in peripheral blood mononuclear cells (PBMCs), occasions specific technical difficulties.

> It has been found that in whole blood cultures, APCs and T cells form adhesive complexes as a consequence of specific antigen activation which prevented detection of responding T cells in cell suspension assays like flow cytometry. The essential modification described herein defines methods for quantitatively recovering small numbers of T cells (<0.1%) from whole blood cultures for analysis from the adhesive antigen-presenting cell (APC) cellular complexes.
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> -- Spec. p. 11, line 25 - p. 12, line 4

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[25] Described at page 1416, col. 2 - p. 1417, col. 1.

The specification further teaches how such technical difficulties may be overcome.

> In order to allow antigen specific reactions to occur in whole blood environments additional modifications were required. Key elements in these modifications were: (1) Slight agitation during the first hour of incubation of whole blood cultures with antigen to improve APC interactions with specific T cells, (2) use of polypropylene culture tubes to more easily displace T cells from adherent APC complexes, (3) direct addition of EDTA to whole blood cultures to optimize recovery of antigen-activated T cells.
>
> -- Spec., p. 17, lines 4 - 10

The Examiner suggests, however, that "the use of EDTA in washing cells . . . has long been used to prevent cell clumping and to reduce background in fluorescence, as known in the art at the time the invention was made or as evidenced by Seon et al. (U.S. Patent No. 5,407,805)." Yet in so suggesting, the Examiner has "defin[ed] the problem in terms of its solution," an impermissible use of hindsight, *Monarch Knitting Machinery Corp. v. Sulzer Morat GmbH,* 45 USPQ2d 1977, 1982 (Fed. Cir. 1998); it was in the ***knowing*** that there was a problem that nonobviousness may properly be found.

Only a tiny percentage of T cells prove recalcitrant to recovery when the method of the present invention is performed in whole blood: as explained in the specification, the problem is "quantitatively recovering small numbers of T cells (<0.1%) from whole blood cultures for analysis." Critically, this small number of adherent cells is <u>the same</u> small number that are specifically activated by the prior incubation with antigen. The result: no observable antigen-specific intracellular cytokine signal (loss of all of the antigen-specific cells) with controls nonetheless

reporting the expected number and percentage of total T cells, of $CD4^+$ T cells, of $CD4^+CD45RO^+$ T cells, of $CD8^+$ T cells. And not only is the result negative, it is exactly as would have been predicted by the prior LDA and ELISPOT data, discussed in some detail above.

Thus, nothing in the prior art would have motivated pursuit of a solution to a problem that could not have been known to exist. From the nonobviousness of the problem flows the nonobviousness of the solution. Applicants respectfully submit that detecting antigen-specific cytokine production by individual T lymphocytes in whole blood samples would have been nonobvious, and respectfully request that the rejection be withdrawn.

Provisional Rejections Over Application Serial No. 08/760,447 Have Been Obviated and Mooted By Abandonment Of The '447 Application

Mar. 1 2 1999

The Examiner has provisionally rejected claims 1, 3 - 6, 8 - 10 and 13 - 17 as previously pending in the present application over claims 1 - 2, 4 - 8, and 9 - 15 of application Serial No. 08/760,447, under 35 U.S.C. § 101, for double patenting. The Examiner has further provisionally rejected prior-pending claims 2, 7, 11, 12 and 18 of the present application over claims 1 - 15 of copending application Serial No. 08/760,447 under the judicially created doctrine of obviousness-type double patenting.

The Examiner has further provisionally rejected claims 2, 7, 11, 12 and 18 under 35 U.S.C. §§ 102(f)/103 and/or 102(g)/103 over the disclosure of copending application 08/760,447, shifting the burden to Applicant to demonstrate that the provisions of 35 U.S.C. § 103(c) apply. Finally, the Examiner provisionally rejects

claims 1 - 18, as previously pending in the present application, over the disclosure of application 08/760,447, under 35 U.S.C. §§ 102(e)/103.

As noted above, the '447 application has been deemed abandoned for failure timely to prosecute. The application will not be revived, obviating each of the above-mentioned provisional rejections as applied to the claims previously pending in the present case, and mooting such rejections as against the claims newly added by amendment herein.

Conclusion

Applicants respectfully submit that the amendments, argument, and evidence submitted herein have fully addressed each of the outstanding rejections, and that the claims, as amended, are in good and proper form for allowance. Applicants invite the Examiner to call the undersigned at Fish & Neave (650.617.4000) if, in his opinion, any matter might more expeditiously be addressed by means of telephonic interview, particularly in light of the large number of procedural papers filed herewith.

Respectfully submitted,

MARCH 5, 1999

Daniel M. Becker (Reg. No. 38,376)
Attorney for Applicants

FISH & NEAVE
1251 Avenue of the Americas
New York, NY 10020
650.617.4000

I hereby Certify that this Correspondence is being Deposited with the U.S. Postal Service as First Class Mail in an Envelope Addressed to: ASSISTANT COMMISSIONER FOR PATENTS WASHINGTON, D.C. 20231, ON

3.5.99

SUSAN B. SNIDER

Signature of Person Signing

Papers Enclosured

- Associate Power of Attorney
- Petition under 37 C.F.R. § 1.137(b)
- Petition under 37 C.F.R. § 1.136(a)
- Petition under 37 C.F.R. § 1.48(c)
- Supplemental Declaration under 37 C.F.R. §§ 1.67(a) and 1.48(c)
- Information Disclosure Statement pursuant to 37 C.F.R. §§ 1.56, 1.97(c), and 1.98, with PTO-1449

Papers Attached

Exhibit A: sheet reproduced from Becton Dickinson Immunocytometry Systems 1998 Product Catalog

Exhibit B: sheet reproduced from the Pharmingen Cytokine/Chemokine Manual (2nd ed., April 1998)

Exhibit C: flow cytometer dot plots, IFN-γ production v. CD69 in the presence of anti-CD28 and anti-CD49d

Exhibit D: flow cytometer dot plots, CD69 v. γ-IFN, TNF-α, and IL-2, with and without addition of anti-CD5 antibody

Exhibit E: Brett *et al.*, "Limiting dilution analysis of the human T cell response to mycobacterial antigens from BCG vaccinated individuals and leprosy patients," *Clin. exp. Immunol.* 68:510-520 (1987)

Exhibit F: Lolli *et al.*, "HIV antigen-reactive T cells detected by antigen-induced interferon γ secretion," *AIDS Res. Human Retrovir.* 10:115 (1994)